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# Physicochemical monitoring of wastewater from a sugar and ethanol industry after bioaugmentation, with a proposal for reuse

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**ABSTRACT.** The sugar and ethanol industry generates large quantities of waste liquids. If untreated effluents are used in fertigation, they might contaminate groundwater and, if they are released into water bodies, they might also jeopardize the survival of the aquatic ecosystem. Therefore, the proposal of this study was to isolate microorganisms from the treatment station of a sugar and ethanol industry, assessing their enzymatic behavior and effects in laboratory, as well as the physicochemical improvement of the effluent. Following a bioaugmentation of the effluent, a physicochemical monitoring was performed with the purpose of reusing it in drip irrigation.

**Keywords:** biorremediation, water quality, wastewater treatment, bioaugmentation.

## Monitoramento físico-químico de águas residuárias de indústria de açúcar e álcool após bioaumentação, com proposta de reuso

**RESUMO.** A indústria de açúcar e álcool gera grandes quantidades de resíduos líquidos. Se estes efluentes não tratados forem utilizados na fertirrigação podem contaminar as águas subterrâneas e, se eles são liberados em corpos d'água, também podem colocar em risco a sobrevivência do ecossistema aquático. Portanto, a proposta do presente estudo foi isolar microrganismos de uma estação de tratamento de indústria de açúcar e álcool, avaliando o seu comportamento enzimático e efeitos em laboratório, bem como a melhoria físico-química do efluente. Foi realizado o monitoramento físico-químico após a bioaumento do efluente com o objetivo de reutilizá-lo na irrigação por gotejamento.

**Palavras-chaves:** biorremediação, qualidade da água, tratamento de águas residuais, bioaumentação.

## Introduction

Although supply failure is a fact in many places, the illusion still exists that water is an infinite resource (Cullet, 2007). Water bodies within densely populated and industrialized areas suffer deterioration processes that compromise their quality. Planning and prevention are necessary to guarantee the present and future use of this resource, with the implementation of sanitary surveillance measures. Selecting parameters of interest to evaluate water quality, however, depends on its source, use and destination (Mounir, Ma, & Amadou, 2011).

For example, special attention must be given to water reuse for agricultural purposes, given the sector's high demand for the resource (some 80% of the available water), because the sustainability of food production will not be kept without new sources and adequate management of conventional water resources (Young & Loomis, 2014).

The myriad uses of water during the industrial production process of a sugar and ethanol agro-industrial plant generate large quantities of liquid waste, which could possibly be reused in a closed circuit equipped with filters, in the initial washing of sugarcane and also in fertigation. Management of the industry's liquid waste (vinasse, wash water from sugarcane, ashes, gases, equipment and flooring, and condenser water) must include special treatment (Santos, Santos, Santos, Santos, & Pacheco, 2009).

In general, the temperature of such untreated wastewater is high at the moment of discharge, preventing the diffusion of atmospheric oxygen, especially if discharged in small lagoons, and low concentrations of dissolved oxygen (DO) are usually detected at floodgates/pumping stations of receiving water bodies where highly contaminant waste water is discharged. Such a drastic reduction of DO at such spots is frequently associated with the death of fish and other water life forms in these watercourses. They also have a high biochemical oxygen demand (BOD) varying from 1700-6600 mg $L^{-1}$, which creates conditions for colonization by anaerobic microorganisms that generate hydrogen sulfide, with a disagreeable characteristic odor (Hampannavar & Shivayogimath, 2010).

They also have high chemical oxygen demand, between 2300-8000 mg $L^{-1}$, with effects on receiving water bodies that are synergistic and similar to those caused by high BOD, allowing the precipitation of iron and other salts, making the water dark and highly toxic to higher organisms. Furthermore, the level of total suspended solids in these waters is generally above 5000 mg $L^{-1}$, leading to the proliferation of plants on the surface of the waterbody, which already presents temperatures around 29-35ºC, consequently increasing turbidity and inhibiting photosynthetic organisms at other depth levels (Jadhav, Vaidya, & Dethe, 2013).

When the BOD/COD rate is higher than 0.6, there is indication of high levels of biochemically oxidizable organic matter, thus the biological process must be employed to treat the wastewater. On the other hand, if this rate is lower than 0.2, the physicochemical treatment must be employed. However, when the relation between BOD and COD is within 0.2 and 0.6, the wastewater may still be treated biologically (Morais & Fonseca, 2013).

Therefore, as a result of the problems of high discharges and low quality of the water from sugar and ethanol plants, as well as the relevance of maintaining our water resources, the present study had the objective of assessing the use of bioaugmentation technology in the wastewater treatment system of S.A. Usina Coruripe Açúcar e Álcool (Coruripe-AL), thus monitoring its physicochemical parameters during the 2004/2005 – 2008/09 harvests.

## Material and methods

### Study site

During the 2003/04 harvest, we collected wastewater samples from the two optional lagoons and the Lateral Uptake canal (Figure 1), which were transported inside a thermal box on ice to the Laboratory of Biochemistry of Parasites and Environmental Microbiology (LBPMA in Portuguese). At the lab, the microorganisms were isolated for identification and selected for use in association in the biological treatment.

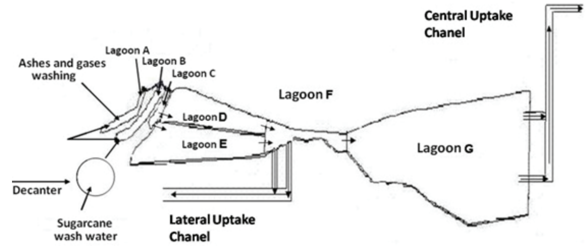


**Figure 1.** Plan of the wastewater treatment station (WTS) lagoons. The wastewater is generated at the headquarters of S.A. Usina Coruripe Açúcar e Álcool, at Coruripe, Alagoas State, Brazil.

### Isolating and identifying the microorganisms

The samples were combined and diluted (1:$10^4$, 1:$10^3$, 1:$10^2$ and 1:10 v $v^{-1}$) in sterile, distilled water and then inoculated (500 $\mu$L aliquots) in a solid culture medium (agar-sugarcane juice 25%) in Petri dishes (9 cm in diameter). The assays were carried out three times. The cultures were incubated at a temperature of 30 ± 2°C, in the dark, for 24-168 hours, and assessed daily with relation to the macroscopic structure of the colonies that appeared. After the various incubation intervals, the colonies were plated out for isolation of the microorganisms initially present and placed in the same medium. From the second plating on, the cultures were placed in different media for biochemical assays and identification.

Different media and staining were used for the identification process by microscopy. Therefore, the isolates were initially separated into gram negative or positive bacteria, endospore-producers or not, alcohol-acid resistant or not, and ascomycetes, yeasts and filamentary fungi. The Gram staining test was confirmed by the method described by Suslow, Schroth & Isaka (1982).

The enzyme activity of the isolates was verified using the methods described by Perovano Filho, Silva & López (2011a). The microorganism isolates were identified according to the bacteria and fungi classification keys (Holt, Krieg, Sneath, & Staley, 1999; Perovano Filho, Silva & López, 2011b).

### Microorganism growth in agrochemicals

Nutrient agar dishes were employed with minimal concentrations of nitrogen sources (1% of the value established in the normal composition of nutritional broth) and 18 different agrochemicals used in the plantations of Usina Coruripe S.A. (Actara, Benomil, Carbofuran, Diuron 800, Etilenox, Fluazifop butil, Glifosato, Hexazinone + Diuron, Imazaphyr, Isoxaflutole, Lambid-cialo trin, MSMA, Paraquat, Plateau 70 DG, Plenum, Sencor 480, Sulfentrazone and 2,4-D-amina) in concentrations of 800 ppm of the active ingredient.

These dishes were inoculated with the wastewater isolates and incubated for 5 days at 30ºC, in the dark.

### Selection of microorganisms for consortium

Three previously isolated bacteria (*Pseudomonas* sp, *Corynebacterium* sp and *Pseudomonas aeruginosa*) and three fungi (*Mucor* sp, *Phanerochaete chrysosporium* and *Geotrichum candidum)* that exhibited satisfactory responses in the determination of enzyme activity and in organic matter and agrochemical degradation tests were selected for antagonism tests with the objective of assessing the possibility of being used in microbial consortia for bioaugmentation.

### Bioaugmentation of the wastewater treatment station of a sugar and ethanol agro-industrial plant

The bioaugmentation of the wastewater treatment station of the agro-industrial plant targeted in this study took place at lagoons 'D' and 'E' (Figure 1**)**, using the selected microorganism consortium. For the inoculation of each microorganism, three Duran-Schott® (1 L) bottles were prepared, each containing 500 mL of a medium with the following optimized composition: 20000 saccharose; 6000 total protein; 3600 total fat; 20 calcium; 6 phosphorus; 1.5 iron; 0.2 copper; 0.3 magnesium; 0.6 zinc; 0.012 iodine; 0.0022 selenium; 2.4 α-tocopherol; 2 ascorbic acid; 0.44 thiamin; 0.14 riboflavin; 0.1 pyridoxine; 0.88 cyanocobalamin; 0.044 folic acid; 0.02 pantothenic acid; 0.96 nicotinic acid; 46 choline; and 2.7 mg $L^{-1}$ biotin.

In parallel, we obtained aqueous suspensions of cells and conidia from cultures (30 ± 2ºC, in the dark) in nutrient agar medium (bacteria, 72 hours) and Sabouraud agar (fungi, 120 hours). After autoclaving (121ºC, 1 ATM, 20 min), the medium in the bottles was inoculated with 5 mL suspension of the respective inoculum ($10^8$ cells $mL^{-1}$ for bacteria and $10^6$ conidia $mL^{-1}$ for fungi). Afterwards, the cultures were incubated for 96 hours (30 ± 2ºC, dark) before being used for reinoculation.

During the harvests, 14 aseptic gallons containing 15 L of bacteria consortium cultures and 14 aseptic gallons containing 15 L of fungi cultures were prepared every fortnight and stored for daily use.

The medium contained in the gallons had the same composition and each gallon was inoculated with 100 mL of the bacteria/fungi cultures. This inoculum volume was calculated with equations established in previous individual studies of the growth kinetics of each of the microorganisms in such a medium, so that they present a cell/conidial concentration corresponding to $1x10^{19}$ (cells or conidia) $mL^{-1}$ on the date of field application.

The inoculated gallons were initially stored in growth chambers (18ºC) and every day, 1 gallon of the fungi consortium and another of the bacteria consortium were removed for application at the pumping station, at the entry of the mentioned lagoons. Furthermore, 6 hours prior to each application, a neutralizing treatment with NaOH (75 kg $application^{-1}$) was carried out at the exit floodgates of the primary decantation lagoons.

### Monitoring the quality of treated wastewater

In order to monitor the effect of the treatment during harvest, wastewater samples were collected every fortnight from the entry of the decantation lagoons ('A' and 'B'), where there was no treatment, and at the exit of optional lagoons 'D' and 'G' of the same WTS, as well as from the central and lateal uptake for irrigation, both channeling waters that had already been through treatment.

Water samples were collected in aseptic bottles and transported to the laboratory in ice, in a polystyrene box. The parameters pH, temperature, DO and electric conductivity were assessed upon sample collection. For that, we used a pHmeter (PHTEK™, model pH-100), a conventional thermometer, an oxymeter (Instrutherm™, model MO-880) and a conductivimeter (Instrutherm™, model CD-840). Further parameters were obtained from assays carried out at LBPMA following techniques described in the manual of the American Public Health Association (American Public Health Association [Apha], 1998).

## Results and discussion

Following the analyses performed on the collected samples, the data obtained during each harvest were transformed into average ± standard deviation. Such values were compared to reference values for Class 3 waters (Conselho Nacional do Meio Ambiente [Conama], 2005).

As regards COD values (Figure 2A) obtained along the WTS in the harvests assessed, we verified an organic matter reduction of around 71 (2004/05), 77 (2005/07), 89 (2006/07), 71% (2007/08) and 90% (2008/09) from lagoon 'A' to 'G'.

We also verified that average COD during the 2005/06 harvest was the highest in all sites assessed, and it occurred due to the removal of the screen that retained excess bagasse pith before the wastewater entered the WTS in this harvest.

Average values observed for this parameter during the harvests assessed remained above the limit (150 mg $L^{-1}$) recommended by legislation in force for Class 3 waters, which does not necessarily render it unfeasible for fertigation (Conama, 2005).

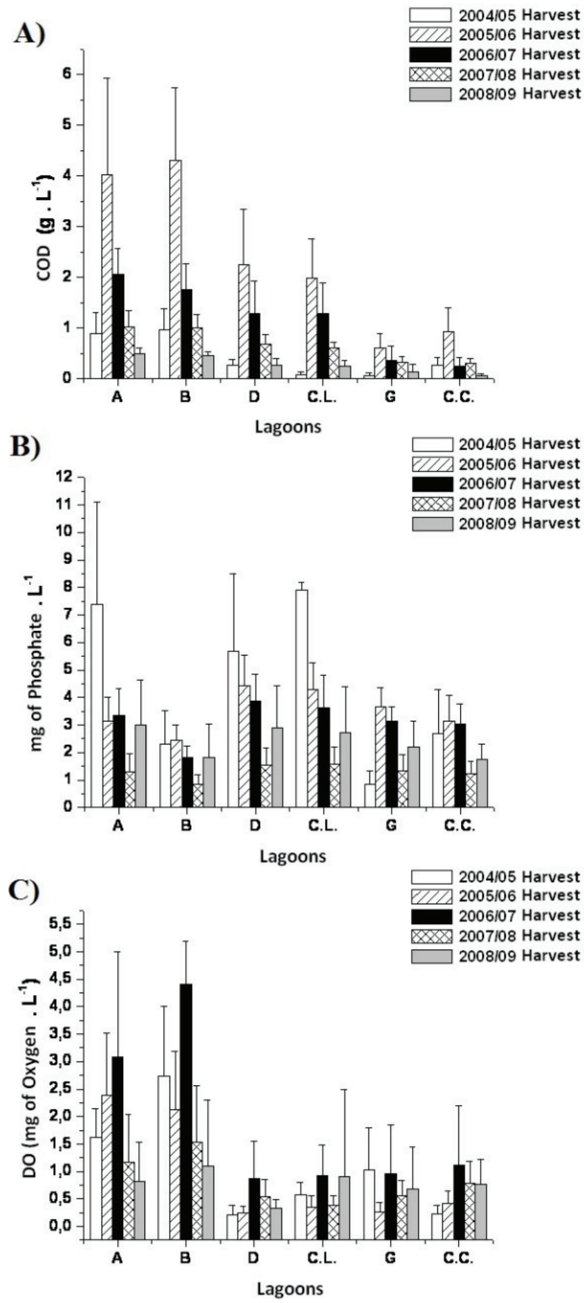


**Figure 2.** Average (± standard deviation) concentration of: A) Chemical Oxygen Demand (COD); B) Phosphate and; C) Dissolved Oxygen (DO) in the waters of the WTS of S. A. Usina Coruripe Açúcar e Álcool, during harvests 2004/05-2008/09, after treatment with bioaugmentation. (A, B = decantation lagoons; D = facultative lagoon; G = last facultative lagoon; C.L. = lateral uptake for irrigation; C.C. = central uptake for irrigation.

High COD values are also indicative of high organic content, biodegradable or not, consuming oxygen dissolved in acid medium, which can cause a number of problems for the receiving bodies if it is dumped without prior treatment. This high inert fraction (COD) in the lagoons also demonstrates the need for improved physicochemical treatment associated with biological treatment, in other words, an improved solid residue removal process at the beginning of the process (Abowei, 2010).

By using an upflow sludge reactor and an anaerobic blanket to assess COD removal in synthetic wastewater based on vinasse constitution with initial COD of 1 g $L^{-1}$, Yassar and Tabinda (2010) found that a minimum hydraulic retention time of 4 hours and an organic matter carrying rate below 6.7 g of COD $L^{-1}$ $day^{-1}$, led to a COD removal efficiency of approximately 76% when the hydraulic retention time was 4 to 6 hours.

When comparing COD removal results reported in this study with those of synthetic wastewater in pilot reactor assays at test benches, all pointed variables considered, it is possible to verify the potential of application of these consortia to remove COD from the wastewater studied.

Phosphate concentrations (Figure 2B) in the wastewater samples treated demonstrated the removal of this parameter along the WTS lagoons. However, such average values were above what was recommended by legislation (0.15 mg $L^{-1}$), indicating the need to remove phosphates or to correct the COD: N: P rate to 100: 5: 1, which is recommended for an optional anaerobic treatment. Monitoring phosphorus concentration is paramount to control the growth rates of algae and cyanobacteria (eutrophication) in water bodies, since they are dependent on this nutrient group (Krishnaswamy, Muthuchamy, & Perumalsamy, 2011).

Even with the high levels of phosphate present in the wastewater during the harvests assessed, the risk of eutrophication of the WTS lagoons studied did not become a fact, since during the study period, such wastewater also displayed very low or barely detectable levels of nitrate and nitrite, which disfavors the eutrophic process (Camargo & Alonso, 2006; Schindler et al., 2008).

The reduction in dissolved oxygen concentrations along the lagoons (Figure 2C) before the final WTS sites (Lagoon G and Central Uptake) during the studied period indicated its consumption in the lagoon where the consortia were applied. Even though the DO concentration increased in Lagoon G, the Central Uptake remained below the minimum limit (≥ 4 g $L^{-1}$) recommended by legislation for Class 3 waters, indicating that a source of artificial aeration would very much favor the treatment.

Only anaerobic bacteria can survive when concentrations of DO are below 2 mg $L^{-1}$. Furthermore, concentrations of DO equal to or even near what is recommended by legislation in force help preventing the formation of substances with undesirable odor, such as ammonia and hydrogen sulphide, for example, from anaerobic biological oxidations (Kartal et al., 2011).

Average temperature values (Figure 3A) of the samples indicated that of the sites assessed, those observed in lagoon A were above recommended (≤ 40°C) by legislation, although in all lagoons this parameter was unfavorable to gaseous exchanges, especially atmospheric oxygen.

Sudden variations in water temperature cause harmful effects to water ecosystems. Temperature increases result in the reduction of dissolved oxygen and in the consumption of oxygen due to the stimulation to biological activities (Naime & Fagundes, 2005).

Although Electric Conductivity does not determine which ions are present in residual water samples, it can help recognizing the impacts that their presence can cause in receiving bodies (Farias, 2006).

The values observed for Electric Conductivity (Figure 3B) in the initial lagoon samples were always above recommended (≤ 750 $\mu$S $mL^{-1}$), indicating the presence of ions from the degradation process; however, a reduction along the WTS was always observed in the samples from Lagoon G and Lateral Uptake.

The pH index (Figure 3C) observed in all lagoons during the harvests studied was always within the limit recommended for Class 3 waters, that is, between 5.0 and 9.0. This index is influenced by the quantity of organic matter to be decomposed, producing humic and/or fulvic acids, or by high concentrations of plants in general, which can cause the medium's acidification as a result of the process of photosynthesis (Moura, Martín, & Burguillo, 2007). According to Araújo & Oliveira (2013), a pH range between 6 and 9 corresponds to the limit to maintain and protect water life, and pH values below 6 or near 5 can solubilize heavy metals, increasing the possibility of toxicity.

As regards Total Dissolved Solids (Figure 3D), their concentrations remained always within the limit range recommended by legislation (≤ 500 mg $L^{-1}$) for Class 3 waters upon analysis of samples from the Central Uptake of harvests 2005/06-2008/09 and from lagoons B, D and G and Lateral Uptake of harvest 2008/09.

Waters that have an excessive concentration of TDS, especially with concentration of minerals above 1000 mg $L^{-1}$, which gives them a disagreeable flavor, are considered as of low quality for a number of purposes. However, if the values encountered are below 500 mg $L^{-1}$, they are considered satisfactory for domestic and industrial use. The sedimentation of organic solids, on the other hand, can create slush deposits at the bottom of WTS lagoons, generating accumulations with short-circuits for the treatment, as well as areas with high CID or no production of gases, and it can also cover small animals, plants and fish eggs (Farias, 2006).

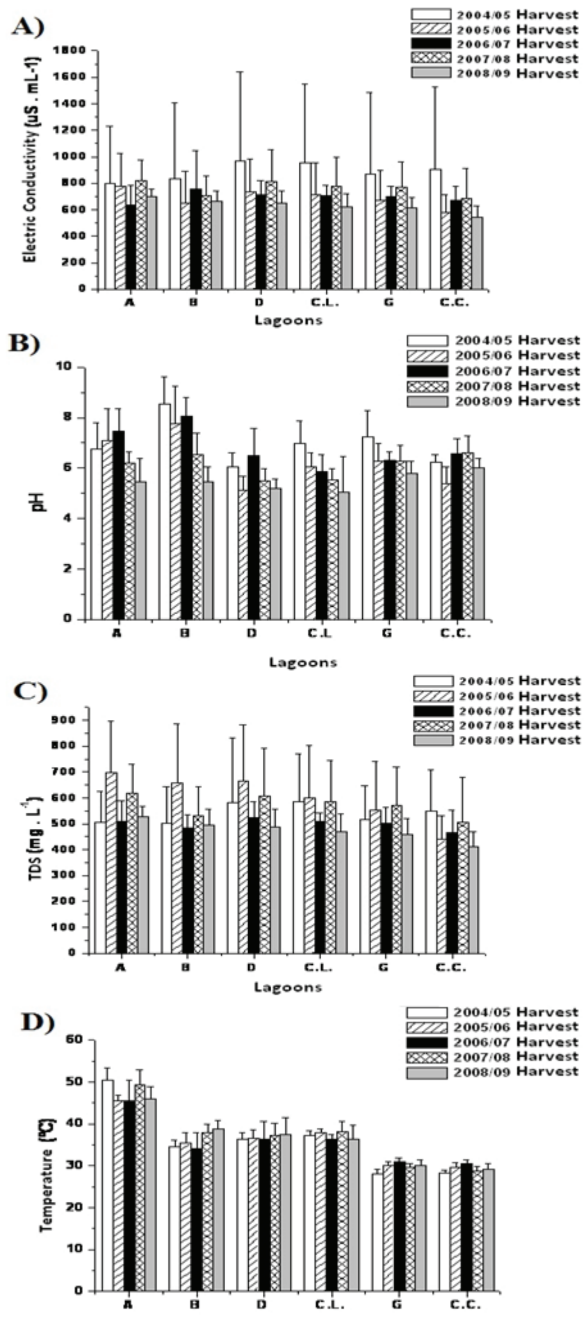


**Figure 3.** Average (± standard deviation): A) Electric Conductivity; B) pH; C) Total Dissolved Solids (TDS) and; D) Temperature, of the WTS waters of S. A. Usina Coruripe Açúcar e Álcool, during harvests 2004/05-2008/09, after treatment with bioaugmentation (A, B = decantation lagoons; D = facultative lagoon; G = last facultative lagoon; C.L. = lateral uptake for irrigation; C.C. = central uptake for irrigation.

Some of the parameters assessed do not yet follow the legislation regarding discharging in rivers (BOD, COD, phosphates), especially with respect to uncontrolled flow, large quantities of residual material gradually accumulating in the lagoons and forming colloids with humic and fulvic acids, and the short-circuits that reduce the volumetric capacity of the lagoons (hydraulic retention time) and hinder the homogenous action of microflora in the bioaugmented environment. However, the purpose of the treatment is to render the water compatible with its use in fertigation, and in this case, the quantity obtained from the Central Uptake is adequate. Although the phosphate levels are still much above the recommended for Class 3 waters, as already mentioned, this does not affect soil irrigation.

The need for physical improvement of WTS lagoons in the refinery studied must be emphasized, especially with regard to more efficient removal of material in suspension (bagasse pith), whether by improved performance sieves (cush-cush with finer screens) or associated with a prior coagulant treatment.

The excess of residues deposited and accumulated along the years must be removed between harvests, with the objective of favoring the implementation of an aerating equipment, especially in Lagoons D and E, favoring the proliferation of aerobic microorganisms capable of surviving in low pH water and clearing the water color, such as some fungi and aerobic bacteria already revealed as promising in *in vitro* kinetic experiments.

## Conclusion

The assessments of quality parameters of the wastewater treated with bioaugmentation using fungi and bacteria consortia indicate that, although a number of variables are constantly being introduced in the field experiment, the biological strategy was considered promising, since it improved the values of a few water quality parameters, such as pH, Electric conductivity, Total Dissolved Solids, COD, temperature and total phosphates along the WTS studied.

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